Factory-Based Permethrin Impregnation of Uniforms: Residual Activity against Aedes aegypti and Ixodes ricinus in Battle Dress Uniforms Worn under Field Conditions, and Cross-Contamination during the Laundering and Storage Process

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The factory-based permethrin coating technique has only recently been developed. Consequently, no data are available on residual activity, laundering, and weathering resistance in impregnated battle dress uniforms (BDUs) worn under military deployment conditions, or on the cross-contamination potential of such uniforms. Herein, factory-impregnated BDUs wornout during military deployment to Afghanistan were investigated for residual permethrin concentration, residual efficacy against arthropod vectors, and cross-contamination during laundering and storage. When compared with BDUs subjected to 50 defined washings using the U.S. Insect/Arthropod Repellent Fabric Treatment method, no significant differences in efficacy were observed against Aedes mosquitoes, but remaining knockdown activity in Ixodes ticks was significantly better in polymer-coated BDUs. BDUs impregnated by the polymercoating method were found to be effective for the life of the uniform, ensuring protection of soldiers in the field from arthropod vectors, while causing less cross-contamination than those treated by the Insect/Arthropod Repellent Fabric Treatment method.

Introduction

mong the increasing number of vector-borne diseases that A are currently emerging or resurging worldwide, few are vaccine-preventable. Although prophylactic drugs are available for malaria, drug resistance is on the increase and spreading rapidly. For this reason, personal protective measures against hematophagous vectors constitute the first line of defense against arthropod-borne diseases in endemic areas. Additionally, arthropod bites can be painfully distracting and can lead to severe secondary infections, dermatitis, or allergic reactions. Along with outdoor workers and travelers, soldiers are at particularly high risk when operating in vector-infested environments. In both past and present military conflicts and deployments, combat strength has been reduced more by disease and nonbattle injuries than by direct combat casualties.² The trend continues in recent armed conflicts, e.g., in Southwest Asia, where the rate of hospitalization from disease was 72% compared with a 4% rate for battle injuries and a 24% rate for other nonbattle inju-

only recently, with the goal of avoiding the toxicological and

logistical shortcomings associated with spraying and/or dip-

ries. In the year 2000, 1,709 human-pathogenic disease agents

were known to science; 156 of these are currently defined as

"emerging" or "reemerging" pathogens, and 114 (= 73%) are

zoonoses, which, by virtue of their number and ubiquity, clearly

constitute a global threat to human health.³ In 26 of 52 retro-

spectively analyzed wars from 480 BC to 2002 AD, vector-borne

diseases like plague, louse-borne typhus, malaria, yellow fever,

relapsing fever, scrub typhus, and visceral leishmaniasis were

either principal or contributing factors affecting overall mortal-

development of topical repellent formulations and residual in-

secticides that can be impregnated into clothing, tents, and

netting.^{5,6} Permethrin, a synthetic pyrethroid insecticide that

combines the essential qualities of repellency, hot feet, knock-

down, kill and residual activity, has been widely used for three

decades as an arthropod contact repellent in fabric impregna-

tion.¹ Although permethrin is characterized by a high level of potency against a wide range of arthropod vectors, multiple

repellent and toxicological effects on arthropods, rapid reactivity, excellent photostability, resistance to weathering, and low

mammalian toxicity, its incorporation into human apparel and peripheral protective devices must be accomplished safely,

while remaining cost-effective and uncomplicated. This last

factor is especially important because permethrin-treated

equipment is likely to be used by thousands of inexperienced

A major advance in protecting at-risk personnel has been the

ity and military success.4

ping methods.

The purpose of the current study was to analyze the contact toxicity and residual activity of polymer-coated factory-treated uniforms worn-out and washed under field conditions during military deployment in Afghanistan to estimate their efficacy against the yellow fever mosquito, *Aedes agypti*, and the castor bean or sheep tick, *Ixodes ricinus*. Additionally, the possible need for reimpregnation cycles and the quantification of cross-

personnel in the military, as well as by tourists, refugees, and disease-threatened populations in the Third World.

To date, three methods for permethrin-based fabric impregnation have been devised: the absorption method, wherein fabrics are individually treated by dipping or spraying; the incorporation method, known also as "Eulanisierung," that uses heat and salt gradients to bind permethrin into wool or silk fibers; and the polymer-coating method, achieved by specific polymerization of permethrin onto fabrics before the tailoring process. This method has been developed and implemented

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Form Approved OMB No. 0704-0188 contamination of permethrin-free fabrics by polymer-coated factory-impregnated uniforms and uniforms subjected to the U.S. Insect/Arthropod Repellent Fabric Treatment (IARFT) method, a commercially available dipping method widely used for the prevention of arthropod bites, were investigated to assess the risks of environmental pollution and increased permethrin exposure to humans when permethrin-treated and untreated uniforms are laundered or stored together.

Materials and Methods

Test Arthropods

Aedes aegypti eggs (Monheim strain) from a continuously (≥40 years) reared colony were obtained from the Bayer Company (Leverkusen, Germany). Eggs were deposited in distilled water and hatched larvae were fed with homogenized Tetramin fish food until adult emergence. A regimen of 27°C, 70% relative humidity, and a photoperiod of 12:12 light:dark was maintained throughout the study. Adults were fed with 10% sucrose solution, and only non-blood-fed female mosquitoes, 5 to 8 days old, were used in permethrin studies. *Ixodes ricinus* nymphs were collected by flagging in natural habitats on Kuehkopf Mountain, Koblenz, Germany, then transported in glass vials at 18°C, 90% relative humidity, and used directly in laboratory tests.

Permethrin Impregnation of Fabric

Factory-based permethrin-impregnated military battle dress uniforms (BDUs) were supplied by UTEXBEL S.A. (Ronse, Belgium). Fabrics were polymer-coated with permethrin (cis: trans = 25:75) after the dyeing process, but before tailoring. This method yielded a theoretical permethrin concentration of 1,300 mg active ingredient (a.i.)/m², and fabrics were dried by in-process heating at 130°C. Uniforms impregnated by the Insect/Arthropod Repellent Fabric Treatment (IARFT) method (Coulston Products Inc., Easton, Pennsylvania) were "dipped" according to the manufacturer's instructions as described elsewhere.

Laundering Procedure for Testing of Cross-Contamination

Freshly permethrin-impregnated fabrics treated by the IARFT and UTEXBEL methods yielded similar theoretical permethrin quantities (1,250 mg/m² and 1,300 mg/m², respectively) and were washed separately under standardized conditions according to the European Norm 26 330:1993/ISO 6,330:1984, described previously. Identical absolute quantities of 7,200 mg permethrin within the wash solutions during standardized laundering with both impregnation methods were achieved by using 1,580 g IARFT and 1,205 g UTEXBEL BDU fabric, each freshly impregnated. Permethrin cross-contamination after one laundering was measured in previously permethrin-free BDU fabrics for trousers (80% cotton, 20% polyester, specific weight 300 g/m²), BDU fabrics for blouses (65% cotton, 35% polyester, specific weight 210 g/m²), and military sport suit fabrics (61% cotton, 39% polyamide, specific weight 270 g/m²). To simulate the worst case, the ratio of treated to untreated fabric was 4:1. Three samples were taken from each tested material for permethrin quantification.

Storage Procedure for Testing of Cross-Contamination

To simulate a worst-case cross-contamination situation, fresh factory- or IARFT-impregnated fabrics were separately

stored dry and at room temperature (20°C) for 6 months at the bottom of cardboard boxes, either directly in contact with permethrin-free BDU fabrics for trousers or military sport suit fabrics, or not in contact with permethrin-free BDU fabrics for trousers. Three samples were taken from each untreated material and tested for further permethrin quantification.

Uniform Use during Deployment

In 2003, fresh factory-impregnated BDUs were provided to the German Contingent of the Implementation Forces Afghanistan (ISAF) within the Kabul area. Deployment time was usually 6 months, although some members of the contingent split their deployment and stayed for 3 months. Uniforms were worn during regular duty in the field and washed every 1 to 2 days by the ECOLOG Company (Kabul) using commercial 6 washing machines and detergents. Uniforms worn-out or damaged after 70 to 100 launderings and collected for disposal were analyzed for quantification of residual amounts of permethrin and remaining residual knockdown activity. Batch 1, containing 10 BDU trousers and 10 BDU blouses, was provided in May 2004; batch 2, consisting of 8 BDU trousers and 5 BDU blouses, was made available in July 2004. All uniform samples were randomly collected.

Permethrin Quantification

Measurement of permethrin in washed and unwashed fabrics was accomplished using the validated method of the Bundeswehr Research Institute for Materials, Explosives, Fuels, and Lubricants. Three samples were taken from different locations on the material and cut into pieces approximately 0.5×0.5 cm to obtain a mean value.

Scanning Electron Microscopy (SEM) Analysis of Permethrin-Treated Fabrics

SEM analysis of treated fabrics was performed as described previously.⁷

Testing Procedures

Plastic test tubes from the WHO insecticide susceptibility kit⁸ were used as described earlier.⁷

Data Analysis

Arthropod tests were replicated 10 times per wash group and impregnation method. Values were reported as mean \pm SD. Differences in mean knockdown time of the repellent formulations tested were analyzed by analysis of variance (ANOVA) or Levene test ANOVA, ANOVA or Levene test F value, and error degrees of freedom (df). The knockdown effect measured was tested against the residual error at the 5% level (statistical significance). The differences between the least-squares means and the p values associated with these differences were computed and compared using a two-sided t test (comparing treatment 7 groups) or one-sided t test (comparing control to treated groups) at the fifth percentile of significance with the SPSS 8.0 program (SPSS Software GmbH, Munich, Germany).

Results

The average residual permethrin quantities (mean \pm SD) of UTEXBEL-impregnated worn-out or damaged BDU trousers

Residual activity in worn-out BDU trousers

140 - Aedes min. 120 ▲··· lxodes 100% knockdown time 100 80 60 40 20 400 600 800 1000 200 a

Residual activity in worn-out BDU blouses

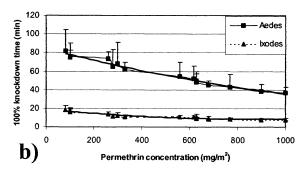


Fig. 1. Permethrin concentration-dependent knockdown activities measured against Ae. αegypti and Ix. ricinus nymphs in BDUs worn-out during deployment: a, trousers; b, blouses.

and blouses during deployment in Afghanistan were estimated to be $454.7 \pm 314.7 \text{ mg/m}^2$ and $482.8 \pm 296.7 \text{ mg/m}^2$, respectively. The permethrin content ranged from 13 to 960 mg/m² in trousers and from 82 to 1,000 mg/m² in blouses, showing no statistically significant differences (F = 0.002; df = 13; p = 0.967). Two trousers (13 and 62 mg/m²) and one blouse (82 mg/m²) showed a permethrin concentration of <100 mg/m² and two trousers (150, 170 mg/m²) and two blouses (both 100 mg/m²) showed permethrin concentrations $\geq 100 \text{ mg/m}^2$, but <200 mg/m².

The resulting permethrin concentration-dependent knockdown activities measured against Ae. aegypti and Ix. ricinus nymphs in BDU trousers and blouses are depicted in Figure 1. In trousers (Fig. 1a), the 100% knockdown time ranged from 36.6 ± 12.6 to 90 ± 34.4 minutes for Ae. aegypti and from 6.67 ± 2.26 to 19.5 ± 4.2 minutes for Ix. ricinus nymphs. BDU blouses (Fig. 1b) gave 100% knockdown times between 37 ± 5.5 and 82 ± 22.9 minutes for Ae. aegypti and between 7.9 ± 1.4 and 18.7 ± 4.54 minutes for Ix. ricinus. Resulting graphs showed no statistically significant differences within the same test arthropod species (Aedes: F = 0.001; df = 14; p = 0.978; Ixodes: <math>F = 0.002; df = 14; p = 0.962). An immediate excitatory effect as well as strong attempts to avoid contact with impregnated fabric could be detected in all test arthropods, regardless of BDU sample or permethrin concentration.

In batch 1, the mean residual knockdown activity on BDU trousers was estimated to be 68.8 ± 35.4 minutes for Aedes and 13.8 ± 4.1 minutes for *Ixodes*; on BDU blouses, comparable figures were 66.2 ± 29.2 minutes for Aedes (trousers vs. blouses: F = 0.23; df = 18; p = 0.639) and 10.5 ± 2 minutes for *Ixodes* (trousers vs. blouses: F = 1.36; df = 16; p = 0.259). In batch 2, the mean residual knockdown activity on trousers was 46.6 \pm 20.5 minutes for Aedes and 10.3 ± 4.3 for Ixodes; on blouses, equivalent figures were 48.6 ± 13.3 minutes for Aedes (trousers vs. blouses: F = 0.7; df = 11; p = 0.417) and 12.2 ± 4.7 minutes for *Ixodes* (trousers vs. blouses: F = 0.021; df = 11; p = 0.888). Differences between batches were not statistically significant (Aedes trousers: F = 1.47; df = 16; p = 0.28; Ixodes trousers: F = 0.076; df = 16; p = 0.786; Aedes blouses: F = 0.224; df = 0.07613; p = 0.224; Ixodes blouses: F = 3.2; df = 13; p = 0.159). Figure 2 depicts the mean residual knockdown activities in postdeployment BDUs compared with activities after 100 launderings by the UTEXBEL and IARFT methods and after 50 launderings with IARFT, these representing the number of launderings after which reimpregnation is recommended. When compared with the results of the UTEXBEL method after 100 launderings under laboratory conditions (38.3 \pm 5.1 minute for Aedes; 15.2 ± 1 minute for *Ixodes*), mean residual knockdown activity measured under deployment conditions was significantly worse in Aedes batch 1 (trousers: F = 5.4; df = 14; p =0.035; blouses: F = 5.2; df = 14; p = 0.039) and batch 2 (trousers: F = 5.0; df = 12; p = 0.043; blouses: F = 5.2; df = 14; p = 0.039), but not statistically different in *Ixodes* batch 1 (trousers: F = 3.03; df = 16; p = 0.101; blouses: F = 2.05; df = 0.10113; p = 0.175) and batch 2 (trousers: F = 1.9; df = 14; p = 1.9) 0.137; blouses: F = 1.2; df = 13; p = 0.28). For the data obtained after 50 washings by the IARFT method (71.5 \pm 12 minutes for Aedes; 27.1 ± 8.5 minutes for Ixodes), no significant differences could be detected when compared with the Aedes knockdown activity of batch 1 (trousers: F = 1.77; df = 13; p =0.206; blouses: F = 1.22; df = 13; p = 0.28) and batch 2 (trousers: F = 0.35; df = 11; p = 0.562; blouses: F = 0.069; df = 0.0698; p = 0.799), but remaining knockdown activity in *Ixodes* was significantly better in UTEXBEL-impregnated BDU batch 1 (trousers: F = 8.47; df = 18; p = 0.009; blouses: F = 32; df = 18; p < 0.0001) and batch 2 (trousers: F = 6.6; df = 16; p = 0.02; blouses: F = 5.2; df = 12; p = 0.04). Postdeployment residual activities of UTEXBEL-impregnated BDUs showed significantly better results in both Aedes and Ixodes when compared with the results obtained after 100 launderings with IARFT (Aedes:

Mean residual knockdown activity

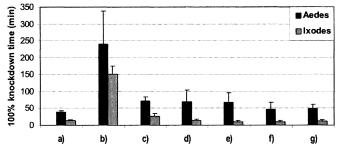


Fig. 2. Mean residual knockdown activities in (a) BDUs after 100 launderings impregnated by the UTEXBEL method, (b) BDUs after 100 launderings when impregnated by the IARFT method, (c) BDUs after 50 launderings when treated by the IARFT method, (d) worn-out BDU trousers batch 1, (e) worn-out BDU blouses batch 1, (f) worn-out BDU trousers batch 2, and (g) worn-out BDU blouses batch 2.

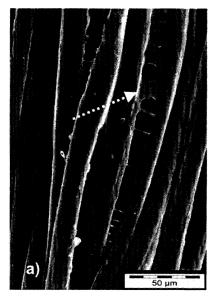






Fig. 3. SEM results for BDU fabric fibers treated by the polymer-coating method: (a) before laundering; (b) worn-out during deployment but with a residual permethrin quantity of 560 mg/m²; and (c) worn-out during deployment and dry cleaned, yielding the measured minimum residual amount of 13 mg a.i. permethrin/m² (—polymer cross-linkages; —damaged surfaces).

batches 1 and 2 trousers and blouses, p < 0.0.001; *Ixodes*: batches 1 and 2 trousers and blouses, p < 0.0.001).

SEM analyses showed that fabrics treated by the polymer-coating method possess smooth-surfaced, polymer-layered, cross-linked fibers before laundering (Fig. 3a); these persist in a worn-out BDU with a permethrin content of 560 mg/m², although disrupted fibers and damaged surfaces are evident (Fig. 3b). However, a BDU trouser worn-out during deployment in Afghanistan and containing the lowest measured amount of residual permethrin, 13 mg/m^2 , showed completely disrupted fibers and dissolved polymer surfaces (Fig. 3c); upon inquiry, it was learned that this uniform had been subjected to dry cleaning as well as intense field wear.

The cross-contamination of untreated fabric laundered with freshly impregnated BDUs treated by the IARFT or UTEXBEL method differed depending on the type of fabric and the treatment method used. When laundered with BDUs treated by the IARFT method, cross-contamination reached 400 mg/m² in BDU trouser fabric, 120 mg/m² in BDU blouse fabric, and 340 mg/m² in military sport suit fabric. Much less cross-contamination was observed with fabrics impregnated by the polymer-coating method: 57 mg/m² in BDU trouser fabric, 32 mg/m² in BDU blouse fabric, and 38 mg/m² in military sport suit fabric (Fig. 4).

Cross-contamination was also observed during storage, with permethrin concentrations ranging from 27 to 65 mg/m² (44 \pm 14 mg/m²) in untreated fabrics that were in direct contact with IARFT-impregnated fabric, whereas UTEXBEL-impregnated fabric produced much lower contamination levels of 10 to 32 mg permethrin/m² (21 \pm 8 mg/m²). Top or bottom layers of untreated fabric showed the lowest amounts of cross-contamination; for IARFT exposure, measurements were 27 mg/m² twice on top, and 35 and 38 mg/m² on the bottom, while UTEXBEL measurements were 10 and 12 mg/m² on top and 15 and 16 mg/m² on the bottom. Untreated fabrics stored with those treated by the IARFT or UTEXBEL methods, but not in contact

with them, showed permethrin concentrations of 0.4 to 2 mg/m^2 and <0.2 to 0.8 mg/m^2 , respectively. In each case, the highest permethrin amounts were recorded in the top fabric layer.

Discussion

The use of type I and type II pyrethroids for personal protection against vector arthropods has been well described and is based mainly on the knockdown effect: locomotor activity disorders and paralysis developing immediately after contact with a toxicant. The knockdown time is one of the critical measures of reliability in an insecticide used as an anti-tick clothing treatment agent, 9 and it is known to be dose dependent. In this study, although the mean value for residual permethrin in worm-out BDUs was relatively high, with amounts >450 mg a.i./m², 11.1% of BDU trousers and 5.5% of BDU shirts showed residual amounts of <100 mg a.i./m², and 22.2% of BDU trousers and 20% of BDU shirts had a remaining permethrin content of <200 mg a.i./m². All but one BDU sample showed values >60 mg a.i./m². Almost no residual permethrin (13 mg a.i./m²) could be detected in one BDU trouser that had been unintentionally

Cross contamination by permethrin-treated BDUs after 1st laundering

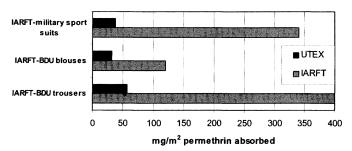


Fig. 4. Cross-contamination in untreated fabric laundered together with BDUs impregnated with the IARFT or the UTEXBEL method directly after treatment.

dry cleaned, leading to almost complete disruption of the polymer-coated surface and simultaneously explaining the exceptionally small residual amount of permethrin in this sample. Although this example demonstrates the importance of proper laundering in maintaining the effectiveness of treated BDUs, even the minimal permethrin remaining on the fibers' surface produced an instant excitatory effect as well as 100% knockdown activity against *Ix. ricinus* nymphs after 19.5 \pm 4.2 minutes. *Aedes* mosquitoes were less susceptible, with a 100% knockdown time of 90 \pm 34.3 minutes, but they also were immediately excited after exposure. No efficacy-dependent minimum permethrin amount serving as a cutoff value for reimpregnation or disposal has been defined to date.

The necessary minimum effective dosage for permethrintreated clothing is chiefly dependent on the vector arthropod family, but other studies have confirmed that even small amounts are effective under laboratory and field conditions. Bed nets impregnated with 80 a.i. permethrin/m² significantly reduce the entry rate of endophilic Anopheles funestus and Anopheles gambiae while simultaneously increasing the exit rate. 10 Laboratory trials conducted with 100 mg a.i. permethrin/m² against the sand fly Phlebotomus papatasii led to a complete cessation of biting activity. 11 Field trials in Belize with bed nets impregnated with 25 to 200 mg a.i. permethrin/m² completely stopped sand fly bites, whether or not the nets were damaged, whereas a control group using nonimpregnated bed nets experienced approximately 10 sand fly bites per person per night.11 Against blackflies and mosquitoes, wide mesh jackets treated with 70 mg a.i. permethrin/m² were reported to rapidly decrease landing counts after the first 10 to 20 minutes of exposure and apparently produced an "area effect," serving to protect both those wearing the treated jackets and others in the vicinity. 12 Additionally, excellent protection from attack by Amblyomma americanum has been reported at dose rates between 62 and 250 mg a.i. permethrin/m². ¹³ For the stable fly, Stomoxys calcitrans, the minimum effective dosage has been determined to be 80 mg a.i./m², causing 100% mortality in 15 minutes following 30 seconds of exposure, while that for the Oriental rat flea, Xenopsylla cheopis, is 320 mg a.i./m² under similar conditions. 14

When compared with worn-out BDUs impregnated by the polymer-coating method, the IARFT dipping method showed similar mean knockdown efficacy against *Ae. aegypti* mosquitoes, but significantly less knockdown activity against *Ix. ricinus* nymphs after 50 launderings carried out under laboratory conditions. Overall, equivalent or better mean efficacy results were obtained with polymer-coated worn-out BDUs than with IARFT-treated BDUs that had reached the recommended point of reimpregnation or disposal. These findings indicate that the remaining efficacy of polymer-coated BDUs worn-out under field conditions is equivalent to that recommended as the baseline for reimpregnation or disposal with the IARFT technique. Assuming that this efficacy is sufficient to protect soldiers against arthropod-borne diseases in the field, polymer-coated BDUs may be regarded as active for the life of the uniform.

An additional problem stemming from individual impregnation of fabrics by dipping or spraying is increased exposure to highly concentrated permethrin solutions and formulations, potentially resulting in increased permethrin incorporation by inhalation or skin contact, thus also increasing the health risk to workers and users.7 With the polymer-coating method, exposure to permethrin is reduced by the higher binding efficacy of the polymer layer on the fiber surfaces. As demonstrated, this technique minimizes cross-contamination of untreated clothing washed or stored with permethrin-treated BDUs. When untreated fabrics are laundered with IARFT-impregnated BDUs, 15% to 50% of the initial permethrin content of the treated material may transfer to the untreated cloth, but this figure falls to only 3% to 5% in the case of the UTEXBEL-impregnated fabrics. Of course, different compositions and specific weights of fabrics, especially variations in cotton content, may result in different levels of permethrin adsorption during the washing process. Even when the polymer-coating formulation decreased the permethrin contamination of untreated clothing over 10 washings, some unavoidable cross-contamination was detected, with permethrin amounts of <80 mg a.i./m² in different fabrics.

During storage, fabrics impregnated by the UTEXBEL method produce less cross-contamination than fabrics treated by the IARFT method, whether contact is direct or indirect. Since permethrin-free fabrics not in direct contact with treated materials also become contaminated, permethrin transfer must occur by air. Therefore, in rooms where permethrin-impregnated BDUs are stored, contamination of dust must be taken into account. If cross-contamination is to be avoided, permethrin-treated BDUs must be washed and stored separately from untreated textiles, and storage rooms must be cleaned regularly.

Our results and those reported in the literature strongly indicate that BDUs washed and worn- out under deployment conditions retain sufficient residual efficacy to successfully protect soldiers from arthropod attack. Additionally, the polymer-coating method is one of the safest and most efficient permethrin treatment procedures currently available. There also remains a need for improvements aimed at increasing the mechanical stability of the coating layer and enhancing the safety and efficacy of the protective formulations.

Conclusions

In an age when vector-borne diseases are emerging or resurging worldwide, personal protective measures are essential for shielding soldiers and other exposed personnel from arthropod attack, especially during deployments in disease-endemic countries. A central focus of personal protection is the use of permethrin-impregnated BDUs. A new polymer-coating technique for the treatment of BDUs has recently been introduced by some NATO- member nations. This technique has been shown to provide residual protection against the entire spectrum of disease-carrying arthopods for the life of the uniform. Polymer coating is also safe and inexpensive, and because the process is completed at the factory, it minimizes logistical problems. Commanders and military medical specialists need only ensure that polymer-coated uniforms are available to their deployed personnel and that such uniforms are properly laundered, thus prolonging their efficacy in force health protection.

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